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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G01N 33/535, 33/567, 30/02 G01N 24/00	A1	(11) International Publication Number: WO 91/07661
		(43) International Publication Date: 30 May 1991 (30.05.91)

(21) International Application Number: PCT/US90/06657

(22) International Filing Date: 20 November 1990 (20.11.90)

(30) Priority data:
438,084 20 November 1989 (20.11.89) US

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(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.

(54) Title: A METHOD OF DETECTING DRUGS IN LIVING AND POST-MORTEM SKIN AND A KIT THEREFOR

(57) Abstract

A method for the detection of one or more drugs in living or post-mortem mammalian skin, which entails: (a) removing a portion of the stratum corneum layer of a living or post-mortem mammal, and (b) detecting the presence of one or more of said drugs from said stratum corneum layer.

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Description

A Method of Detecting Drugs in Living And Post-Mortem Skin and a Kit Therefor

Technical Field

The present invention relates to a method of detecting drugs in living and post-mortem skin and a kit therefor.

Background Art

By all accounts, the use of both legal and illegal drugs has become ubiquitous. Unfortunately, the increased use of certain drugs has been responsible for an increase in the number of drug-related deaths. For example, in one series of 247 randomly chosen cases inclusive of all causes and manner of death that were investigated by a medical examiner's office of a metropolitan area, 35% were positive for drugs in the psychoactive prescription drug category. See Interpretive Toxicology, by J.C. Garriott, Clinics and Laboratory Medicine, Vol. 3, No. 2, June 1983 pp. 367384. The greatest number of such deaths typically result from drug intoxications involving barbiturates, tricyclic antidepressants, propoxyphene and narcotics such as heroin, cocaine and meperidine.

In addition to a rising concern for the increased number of deaths resulting from drug use, there is also an increased concern in the business community regarding the effect of so-called recreational drug use upon safety and

productivity in the workplace. As a result of this concern, routine drug testing is becoming more widespread.

Presently, the particular mode of drug testing which is used may depend upon whether the user is tested alive or post-mortem. For example, if the drug user is tested while living for the presence of one or more drugs, it is most common to test the blood. In fact, blood is the conventional specimen of choice and is typically drawn in sufficient quantity for toxicological analysis. For most laboratories, this means that approximately 15 ml of uncoagulated whole blood is required if analysis for unknown agents is necessary. When using gas and/or liquid chromatographic detection procedures, this amount is adequate for a relatively complete analysis for prescription and abused drugs, as well as for alcohols.

By contrast, if post-mortem analysis is conducted, a variety of autopsy specimens may be used depending upon the circumstances. Although blood may be used in post-mortem analysis, it may be necessary to analyze bile and urine for narcotic identification inasmuch as the drug sought may be present in these specimens in much higher quantities than in the blood, especially if the administration of the drug occurred some hours prior to death.

Regardless of whether a living or post-mortem analysis

is conducted, however, a number of drawbacks exist with conventional analytical procedures. For example, when using blood as an analytical specimen, caution must be used in the collection and preservation of blood. If serum separation tubes are used, the gels therein may remove most of the agent sought in the blood. Moreover, some gels used also contain components that interfere with spectrophotometric absorption or gas chromatographic assays.

Additionally, considering that drug abusers may present a greater risk of infection by the human immunodeficiency virus (HIV), it would be extremely desirable to be able to avoid the use of blood in testing for drugs.

Furthermore, even if the analytical procedure to be used is optimized so as to minimize interference with the drug to be tested, many drugs deteriorate or are lost completely when the human body decomposes.

In practice, many different body compartments have been used for toxicologic and forensic analysis. For example, such diverse compartments as the blood, bile, urine, gastric contents, brain, liver muscle, spinal fluid, spleen and vitreous humor have been used. The tissue sample used depends on a variety of factors, including mode

of administration. It is known, for example, that drugs administered by injection accumulate in the kidneys.

However, the skin has been overlooked as a compartment in drug detection and distribution studies. Except for the pharmacokinetic analysis of griseofulvin, no systematic consideration of drug detection in skin, following exogenous exposure, has been investigated.

Clearly, a need continues to exist for a method which utilizes a reliable body compartment for the detection of a wide variety of drugs, and which is, at once, effective for detecting drugs in both living and post-mortem samples. Furthermore, it would be extremely desirable to attain such a method, which also is highly accurate with a reduced incidence of false positive and false negative results.

Also, it would be extremely desirable to attain such a method, which is also non-invasive and nonintrusive in nature. Ideally, such a method would be non-invasive in not requiring blood samples, and nonintrusive in not requiring urine samples.

Disclosure of the Invention

Accordingly, it is an object of the present invention to provide a method for detecting drugs in living and post-mortem samples.

In particular, it is an object of the present invention to provide a method for detecting drugs in the stratum corneum skin layer of living and postmortem subjects.

Moreover, it is also an object of the present invention to provide one or more kits for practicing the above method.

Furthermore, it is a particular object of the present invention to provide a method for detecting drugs in the stratum corneum skin layer of living and post-mortem subjects which is non-invasive and nonintrusive and has a reduced incidence of false negatives and false positives.

Accordingly, these objects and others are provided by a method for detecting one or more drugs in the stratum corneum layer of living or post-mortem mammalian skin, which entails:

a) removing a portion of the stratum corneum skin layer of living or post-mortem mammalian skin, and b) detecting the presence of said one or more drugs in or from said stratum corneum skin layer.

Brief Description of the Invention

Figure 1 illustrates the detection in an autopsy

specimen of cocaine metabolite and phencyclidine (PCP) using a stratum corneum sample. The internal standard is also shown.

Figure 2 illustrates the detection in an autopsy specimen of cocaine, cocaine metabolite and phencyclidine (PCP) using a stratum corneum sample. The internal standard is also shown.

Best Mode For Carrying Out the Invention

The science of toxicology has significantly grown during recent years, by and large, due to the advancements made in analytical methodology and the interpretation of drug concentrations and chemical effects in the body. With the development of sensitive instrumentation and methodology for using the same, it has become possible to detect and identify minute quantities of drugs and metabolites thereof in human blood or tissue. Although many different tissue specimens have been used for toxicologic examination, both living and post-mortem, it is quite surprising that the skin has been overlooked, if not routinely ignored, as a basis for drug detection and evaluation.

It is known that human skin can act as a reservoir for certain drugs. For example, studies relating to

griseofulvin levels in the stratum corneum layer of the skin have been conducted. However, after discontinuing use of griseofulvin, the drug concentration was observed to fall off more rapidly in the skin than in blood. Thus, it would seem in view of this study that the skin would not be suitable for use in forensic analysis over an extended period of time.

Studies have been conducted using full thickness skin, i.e., about 500 gm, wherein it has been determined that chemical substances can migrate outwards from within the body to the skin surface by diffusion from cutaneous capillaries across the epidermis. However, the use of only the stratum corneum layer of the skin for detecting drugs in both living and post-mortem subjects has never been proposed.

The skin is composed of two distinct structures the dermis, a connective tissue layer covered by the epidermis, and the epidermis which is an epithelial layer. Each of the layers confers special properties on the skin. The dermis provides mechanical strength to the skin by virtue of the presence of collagen and elastic fibers and provides a reservoir of defense and regenerative capabilities. The primary role of the epidermis is to function as the primary barrier to mechanical damage, microbial invasion and desiccation.

The epidermis is non-vascular and consists of stratified epithelium. As noted above, the epidermis forms a defensive covering on the surface of the true skin, and limits the evaporation of water vapor from its free surface. The more superficial layer of epidermal cells, forming a horny layer, is called the stratum corneum.

In greater detail, the epidermis consists of several distinct layers of epithelial cells agglutinated together and having a laminated arrangement. These several layers may be described as composed of four different strata from within outward: (1) the Stratum Malpighi, (2) the Stratum granulosum, (3) the Stratum lucidum and (4) the Stratum corneum. Due to the development of fresh layers underneath, the cells of the Stratum corneum assume a flattened form from the evaporation of their fluid contents. The cells contain no discernable nucleus. See Gray's Anatomy, (15th Edition, 1977).

Quite surprisingly, in accordance with the present invention, it has been discovered that the stratum corneum layer of the skin, by itself, can serve as a useful sample source for the detection of drugs in either living or post-mortem mammalian subjects. That is, the stratum corneum, by itself, suffices as a drug reservoir and it is, therefore, no longer necessary to utilize full thickness skin samples in drug testing.

In general, the method of the present invention may be practiced with either living or post-mortem mammalian subjects. For example, the present method may be easily applied in the toxicological analyses of human bodies which are in an advanced stage of decomposition. Alternatively, the present invention may be used in the routine drug screening of living subjects for a variety of purposes such as workplace drug testing or the monitoring of rehabilitated criminal abusers.

The stratum corneum skin specimens may be obtained from anywhere on the hairless regions of the body. Typically, a portion of stratum corneum may be scrapped off the skin surface, after the removal of hair if necessary, with a rough-edged object or one or more pieces of adhesive material of any variety may be applied to the selected area of the stratum corneum in order to strip a sample from the stratum corneum. Commonly, the scrapping or stripping is conducted anywhere from 1 to 10 times with from 1 to 10 different pieces of adhesive material for stripping. More commonly, the scrapping or stripping of the stratum corneum occurs about four times with four different sheets.

After scrapping off or peeling off the stratum corneum samples, the adhesive material containing the stratum corneum is cut into strips and then a suitable organic solvent, and a suitable buffer and an internal standard is

added thereto. Alternatively, the scrapped sample may be added to this mixture. Then, the mixture is agitated for anywhere from 30 seconds to 5 minutes. Thereafter, the drug to be analyzed is extracted from the mixture, wherein after an alkaline substance is added to the extracted drug in order to form the free base-of the drug.

In general, any organic solvent can be used which is capable of dissolving the drug or drugs to be detected. Generally, solvents such as diethyl ether, methanol, acetone or chloroform are used. However, any solvent which is capable dissolving the drug of interest may be used. Such solvents are well known to those skilled in the art and can be ascertained from the Merck Index, for example. Further, any alkaline substance may be used in an aqueous solution provided that it is capable of releasing the free drug base. Examples of such an alkaline substance is sodium and potassium carbonates and bicarbonates. Then, the drug is extracted from the alkalized-mixture using a suitable organic solvent. Further, any buffer having a pH of about 7.5 to 9.0 may be used.

Thereafter, the drug is analyzed by a number of different analytical techniques, one of--which is gas chromatography/mass spectrometry. However, other techniques may be used such as thin-layer chromatography and immunoassays such as enzyme immunoassays and

fluorescence immunoassays. Enzyme-based assays are of particular interest in the present invention.

For example, numerous methods have been developed in recent years for the detection of drugs as described in U.S. Patents 3,766,162; 3,775,536; 3,799,741; 3,882,245; 3,843,696; 3,853,987; 3,867,366; 3,878,137; 3,879,262; 3,884,898; 3,888,864; 3,952,091 and 3,966,744 all of which are incorporated herein in the entirety.

Of particular interest, however, are assays using antibodies developed for the detection of specific drugs, and assays using cell lines possessing opiate receptors.

For example, antibodies such as those disclosed in U.S. Patents 4,151,268 (barbituric acid and derivatives thereof), and 4,197,237 and 4,123,431 (cocaine and derivatives thereof) may be incorporated onto the surface of a glass slide or adhesive material and then used in the present process to detect the presence of cocaine or barbituric acid or derivatives thereof using reagents as disclosed therein. U.S. Patents 4,151,268, 4,123,431 and 4,197,237 are incorporated herein in the entirety. Notably, either monoclonal or polyclonal antibodies may be used.

Furthermore, antibodies against other drugs are known

to those skilled in the art and may be used in accordance with the present invention as described in the incorporated U.S. patents noted above.

Also, it is possible to utilize opiate receptors in conjunction with a known radio labelling technique in order to detect the presence of one or more drugs in the stratum corneum sample. In this respect, U.S. Patent 4,257,773 is incorporated herein in the entirety.

The method of U.S. Patent 4,257,773 is particularly advantageous as it may be used to detect a very wide variety of drugs.

If antibodies are used in the detection of drugs, the stratum corneum sample may be worked up as described previously and the extracted drug containing solvent mixture may be applied to the antibody-containing surface and then detected using the techniques described in any of the incorporated U.S. Patents 4,151,268, 4,123,431 and 4,197,237.

If opiate receptors are used, the same can be obtained from synaptic membranes of mammalian brain tissue in accordance with known techniques. The opiate receptors may be adhered to a solid support, such as a glass plate or glass beads or the wall of a container which is compatible

with a scintillation counter. The drug or drugs present may then be detected using the technique described in U.S. Patent 4,257,773.

Furthermore, in accordance with another aspect of the present invention, the presence of one or more drugs may be detected using any of the above-described detection means without extracting the same from the stratum corneum samples. In this case, the detecting antibodies, either monoclonal or polyclonal, may be adhered to the surface of the adhesive material used to extract stratum corneum samples in any manner such that stratum corneum samples may be adhered to the surface antibodies.

The same technique may be used, except that opiate receptors or enzyme-containing materials may be adhered to the surface of the adhesive material.

In adhering the antibodies, opiate receptors or enzyme-containing materials, or even enzymes themselves, to the surface of the adhesive material, any substance may be used which effectively binds the same to the adhesive material. Thereafter, the presence of one or more drugs may be detected using any of the standard methods of detection discussed above. For example, well known fluorimetric and colorimetric techniques may be used.

Any of the above procedures are extremely expeditious inasmuch as many samples can be routinely analyzed in only several hours.

Moreover, the present invention is extremely advantageous inasmuch as a wide variety of drugs can be analyzed using the same. For example, drugs which can be detected using the present method are as diverse as nicotine, caffeine, heroin, cocaine, diphenhydramine, lidocaine, meperidine, morphine, quinine and phencyclidine. However, if heroin has been used, morphine is actually detected in the body. In any event, the present method is effective for drugs having a wide disparity of octanol/water partition coefficients and molecular weights.

Having generally described this invention, a further understanding will be obtained by reference to certain specific examples which are provided herein for purposes of illustration only and are not intended to be limiting.

EXAMPLES 1-44

The following examples are provided as illustrating the full thickness skin technique and results obtainable therefrom. However, the examples are only for purposes of illustration and are not intended to be limitative.

In examples 1-44, the following procedure was used for

post-mortem analysis.

Anterior torso skin specimens were obtained from the hairless regions (by showing the area if necessary), along a "Y" shaped incision. The skin specimens were trimmed of grossly visible adipose tissue, with surgical scissors. After removal of subcutaneous fat the specimens were kept at -10 to -20°C. Storage time prior to extraction varied from 4 hours to 4 months. Following freezing the specimens were cut into 0.5 cm cubes. The dissected samples were blended in 20 ml of distilled water for 2 minutes. The blended mixture was placed in a 1000 ml flask. 150 ml of ether, 1.0 ml of AMP (alkaline mono phosphate) such as sodium or potassium mono phosphate buffer and 1.0 ml of internal standard were added. The sample and reagents were agitated for 3 minutes. After shaking, the ether layer was poured into a 150 ml erlenmeyer flask. Add 5.0 ml of buffer (pH 8.5) and shake for 3 minutes, decant and disregard the aqueous layer and repeat the addition of the aqueous buffer solution two more times. Add 3.0 ml of 0.75N H_2SO_4 to the ether, shake for 3 minutes, decant the layer aqueous layer into a glass centrifuge tube. The aqueous solution is then tested for fluorescence with an ultraviolet light.

25 ul from each aqueous sample was used for morphine analysis. Morphine quantitation was performance using the

abuscreen kit from Roche Diagnostics. The remaining aqueous extraction was prepared for GC/MS analysis by adding 0.15 ml of chloroform, 5 drops of KOH and 5 drops of AMP buffer, then vortexed and the pH was tested. The pH should be about 9.0. Then, the aqueous extraction was centrifuged at 8,000 to 10,000 GS for 1-minute. 100 ul aliquot of the lower chloroform layer was removed for GC/MS analysis of cocaine and phencyclidine. GC/MS parent drug identification was based on the detection of all ions, i.e., cocaine: 303, 82 and 182; and phencyclidine: 243, 242, 186 and 200.

Forty-four subjects were analyzed for cocaine. Two were in an advanced stage of decomposition with desiccation of the skin. These are cases 463 and 616. One desiccated sample displayed moderate decomposition with epidermal-dermal separation (case 672) while the remainder showed no gross sign of decomposition. The skin cocaine concentration results are shown in Table 1, along with the blood concentrations. The gas chromatography/mass spectrometry cocaine analysis only detected the parent drug. The skin and blood results were statistically analyzed using Baye's rule. See Tables 2 and 3. Four of the 44 cases were not included in the predictive value analysis for lack of adequate history. Cases 463, 566, 592 and 832 are John Does.

The 16 morphine cases were all from decedents with a positive history of drug use. See Table 4. Blood from case 463 was not available due to decomposition. All morphine concentrations were obtained by radioimmunoassay. Statistical analyses for morphine was also carried out using the predictive value tables. See Tables 5 and 6.

Notably, other drugs were detected. The drugs identified in the study varied in their physicochemical properties. See Table 7. Despite the differences in their chemical properties diffusion into an extraction from the epidermis and dermis was possible. Not only was there variation in the chemical nature of the drugs, but the variation also was present in the circumstances surrounding the deaths and condition of the body. For example, cocaine and phencyclidine (PCP) were found in mummified cases. Diphenhydramine was found in a diphenhydramine overdose showing moderate decomposition, i.e., epidermal-dermal separation. Lidocaine was found in decedents who received bolus lidocane injections during resuscitation and quinine in heroin abusers.

EXAMPLES 45-46

Two studies were then conducted using only the stratum corneum for analysis. The following procedure, i.e., the Hill procedure, was used.

The upper backs of the subjects were lightly washed with tap water and blown dry. Nine inch square sheets of adhesive tape were firmly applied to the back. Each sheet was removed and reapplied four times. Four sheets were used for each Case. The adhesive sheets were cut into several rectangular strips and place in 1000 ml flasks. To the 1000 ml flasks were added 150 ml of ether, 1.0 ml of AMP buffer and 1.0 ml of internal standard. The sample and reagents were agitated for 3 minutes. After shaking, the ether layer was poured into a 150 ml erlenmeyer flask. Then 5.0 ml of buffer (pH 8.5) was added and then agitated for 3 minutes. Thereafter, the aqueous layer was decanted and discarded. The addition of aqueous buffer was then repeated two more times.

Where possible, blood samples were also analyzed to provide a correlation with skin samples. However, blood samples were not always available due to decomposition changes. Analysis was conducted by gas chromatography/mass spectrometry. The skin and blood results were statistically analyzed using Baye's rule.

COCAINE

CASE	SKIN(NG/ML)	BLOOD (NG/ML)
362	67.2	136.6
369	0	0
371	42.8	0
374	82.0	0
375	53.9	0
382	434.4	13.45
390	0	0
401	0	0
407	0	0
438	13.3	0
463	9.2	N/A
490	0	0
492	1328.5	0
511	301.0	0
535	1177.0	0
539	69.0	58
555	3411.7	2187
561	608.0	4.0
562	1675.0	0
566	499.0	0
567	329.5	17.56
582	507.5	146.0
590	40.5	0
592	953.0	148.0
593	0	0
597	70.0	0
611	3.78	0
612	0	5.7
616	56.2	N/A
652	82,512.36	130.0
672	108.7	103.6
680	778.8	68.5
703	306.0	47.7
707	0	0
733	112.0	0
769	181.0	0
812	0	0
827	39.2	0
832	0	95.50
833	1389.9	180.0
841	375.9	50.0
855	0	0
887	1361.4	0
900	310.8	0

TABLE 1

COCAINE

	(+) DRUG HISTORY	(-) DRUG HISTORY
SKIN (+)	30	0
SKIN (-)	8	2
TOTALS	38	2

SENSITIVITY = 78.9 %
SPECIFICITY = 100 %

TABLE 2

	(+) DRUG HISTORY	(-) DRUG HISTORY
BLOOD (+)	14	0
BLOOD (-)	23	2
TOTALS	37	2

SENSITIVITY = 37.8 %
SPECIFICITY = 100 %

TABLE 3

MORPHINE

CASE	SKIN (ng/ml)	BLOOD (ng/ml)
483	98.0	0
463	82.5	N/A
490	15.1	0
511	193.6	70.0
539	201.6	1700.0
561	784.4	1100.0
562	409.5	23.0
592	52.36	0
703	89.3	1030.0
711	92.0	30.0
733	147.9	10.0
764	352.0	640.0
812	261.0	210.0
827	220.0	0
831	496.4	1000.0
841	132.6	230.0

TABLE 4

MORPHINE

	(+) DRUG HISTORY	(-) DRUG HISTORY
SKIN (+)	16	0
SKIN (-)	0	0
TOTALS	16	0

SENSITIVITY = 100 %
SPECIFICITY = 0-100 %

TABLE 5

	(+) DRUG HISTORY	(-) DRUG HISTORY
BLOOD (+)	11	0
BLOOD (-)	4	0
TOTALS	15	0

SENSITIVITY = 73.3 %
SPECIFICITY = 0-100 %

TABLE 6

PHYSICAL-CHEMICAL PROPERTIES OF DRUGS IDENTIFIED

DRUGS	MOLECULAR WEIGHT DALTONS	OCTANOL/WATER PARTITION COEFFICIENT
COCAINE	303	2.34
DIPHENHYDRAMINE	255	
LIDOCAINE	234	
MEPERIDINE	247	1.6
MORPHINE	303	0.1
NICOTINE	162	15.8
PHENCYCLIDINE	243	
QUININE	378	1.8

TABLE 7

In more detail, Table 1 evidences the result (in mg/ml) of cocaine detected in blood and skin samples of the forty-four subjects tested as described above.

Tables 2 and 3 illustrate the high sensitivity and specificity of the present method for the detection of cocaine. Notably, the use of the stratum corneum, in accordance with the present invention, affords a much greater sensitivity than the use of blood.

Table 4 evidences the result (in ng/ml) of morphine detected in blood and skin samples of the forty-four subjects tested as described above.

Tables 5 and 6 illustrate the high sensitivity and specificity of the present method for the detection of morphine. Notably, as with cocaine, the use of the stratum corneum, in accordance with the present invention, affords a much greater sensitivity than the use of blood.

Table 7 lists the molecular weight and octanol/water partition coefficients of a few of the drugs which may be detected by the present method. Notably, the present method is operable in detecting drugs having a variety of molecular weights and octanol/water partition coefficients.

Thus, in accordance with the present invention, it has

now been discovered that the stratum corneum can be used for both living and post-mortem analysis.

Finally, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.

The present invention may even be used for purposes other than the detection of drugs. For example, in accordance with the present invention, stratum corneum samples may be used to detect the presence of bacteria and viruses, in particular the HIV virus.

Additionally, the present invention may be used to detect any endogenous substance such as glucose, sodium and potassium ions, chloride ions, proteins.

The detection of proteins is important as proteins encompass many diverse substances such as enzymes and hormones.

Further, the present invention may also be used to detect steroids.

Finally, in accordance with the present invention is

provided one or more kits for practicing the present invention.

In particular, any standard kit for the detection of one or more drugs may be used in accordance with the present invention provided that either a means for scrapping stratum corneum samples or adhesively removing stratum corneum samples are provided in the kit.

Additionally, the kit of the present invention may include adhesive material for removing stratum corneum samples to which is adhered antibodies (monoclonal or polyclonal), opiate receptors or enzymes or enzyme--containing materials. In essence, one of these detecting means may be immobilized on the surface of the adhesive material. A protective, substantially non-adhesive coating or cover may be placed over the immobilized detecting means until it is ready for use.

In addition to the above, the kit of the present invention also contains various fluorimetric or colorimetric reagents in order to complete the analytical procedure as are well known to those skilled in the art.

Finally, although the present invention may be used advantageously with humans, it may also be used in numerous veterinary applications. For example, it may be used in

conjunction with dogs and horses, such as in racing.

Claims

1. A method of detecting one or more drugs in the stratum corneum skin layer of living or post-mortem mammalian skin, which comprises:

a) removing a portion of the stratum corneum layer of a living or deceased mammal, and

b) detecting the presence of one or more of said drugs in or from said stratum corneum layer.

2. The method of Claim 1, wherein said portion of said stratum corneum layer is removed by scrapping the same with a scrapping means.

3. The method of Claim 1, wherein said portion of said stratum corneum layer is removed by applying an adhesive material to said stratum corneum layer and removing the same therefrom.

4. The method of Claim 1, wherein said one or more drugs in said stratum corneum layer are extracted therefrom prior to detection.

5. The method of Claim 1, wherein said one or more drugs are detected by gas chromatography/mass spectrometry.

6. The method of Claim 1, wherein said one or more drugs are detected using antibodies.

7. The method of Claim 1, wherein said one or more drugs are detected by radioimmunoassay.

8. The method of Claim 3, wherein said adhesive material has monoclonal antibodies, polyclonal antibodies, opiate recepts or enzymes immobilized thereon in an amount sufficient to detect said drugs.

9. An assay kit for detecting one or more drugs in the stratum corneum skin layer of living or postmortem mammalian skin, which comprises:

a) an adhesive material for removing a sample of said mammalian stratum corneum skin layer, from said mammal, having immobilized thereon monoclonal antibodies, polyclonal antibodies, opiate receptors and enzymes, and

b) means for detecting the presence of a conjugate of said one or more drugs and said immobilized monoclonal antibodies, polyclonal antibodies, opiate receptors and enzymes.

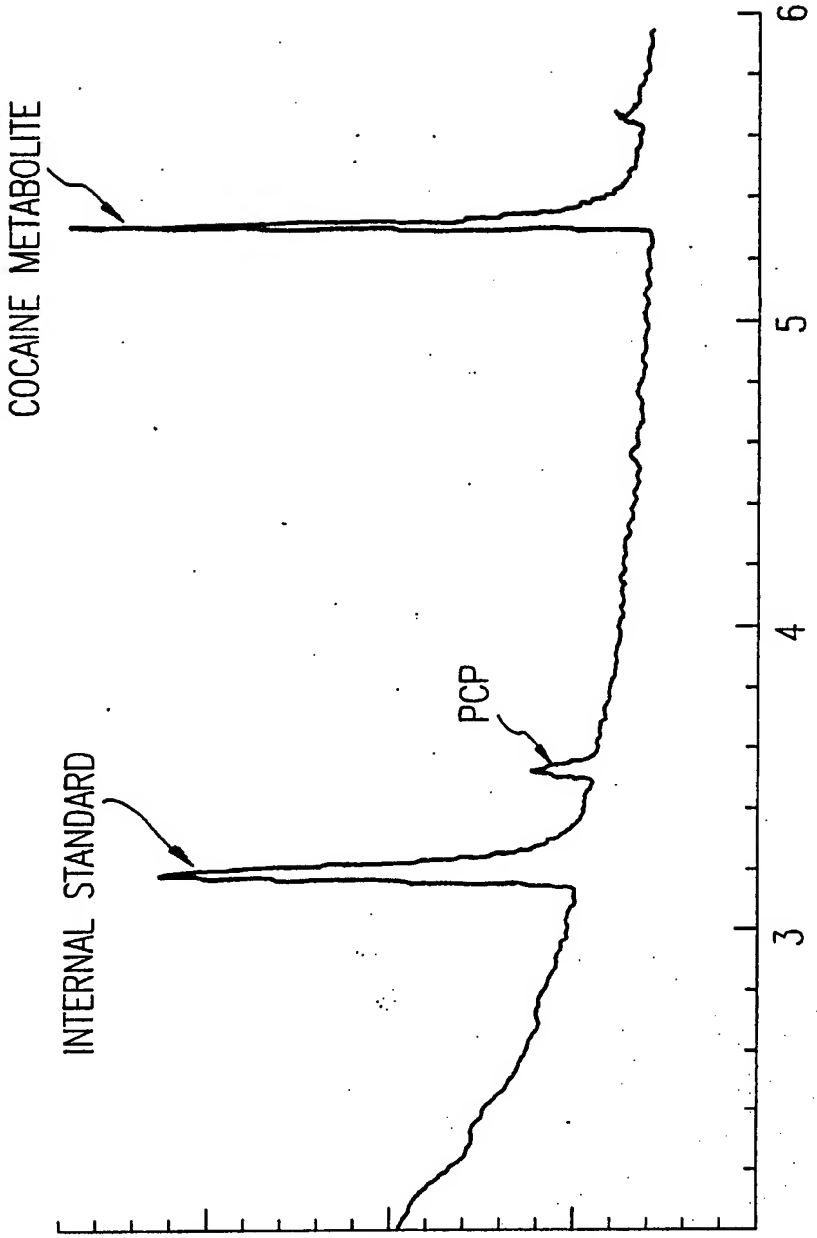
COCAINE METABOLITE
INTERNAL STANDARD
PCP
ABUNDANCE
3.0E4
2.0E4
10000
3
4
5
6
TIME (MINUTES)

FIG. 1A

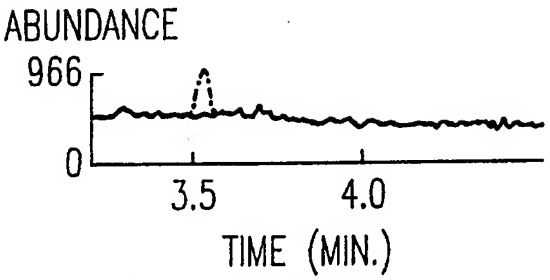

FIG. 1B

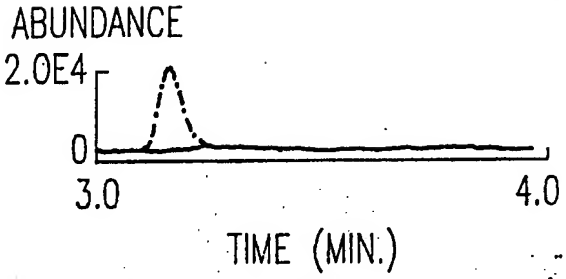

FIG. 1C

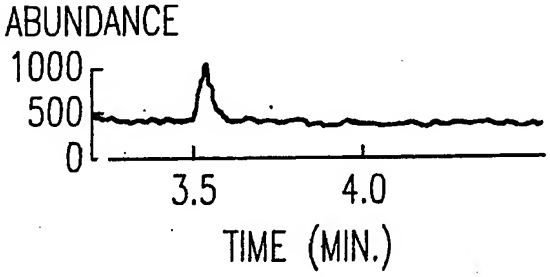

FIG. 1D

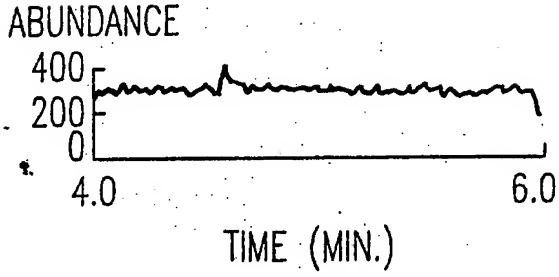

FIG. 1E

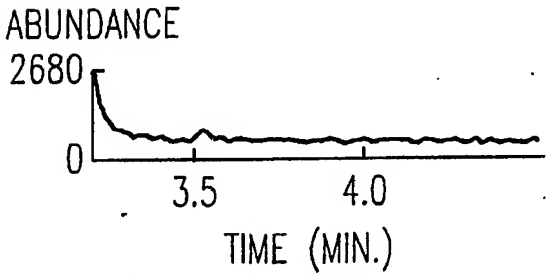

FIG. 1F

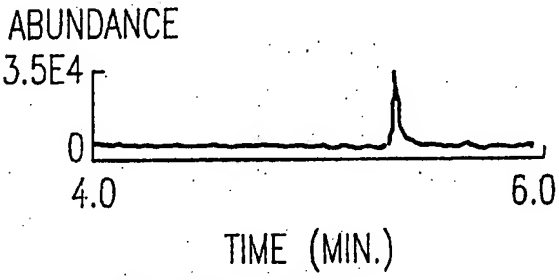

FIG. 1G

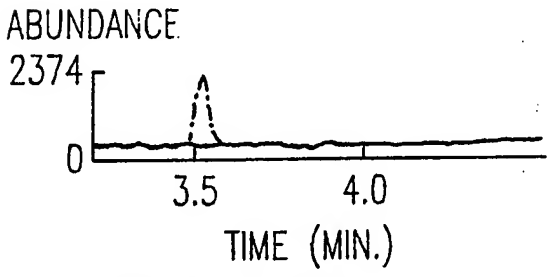

FIG. 1H

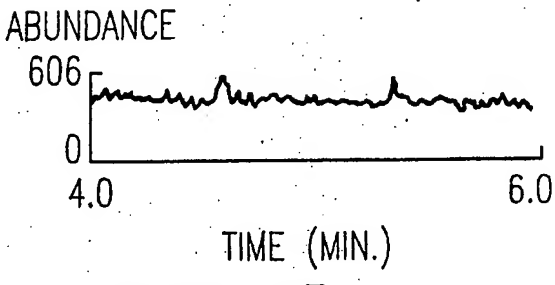

FIG. 1I

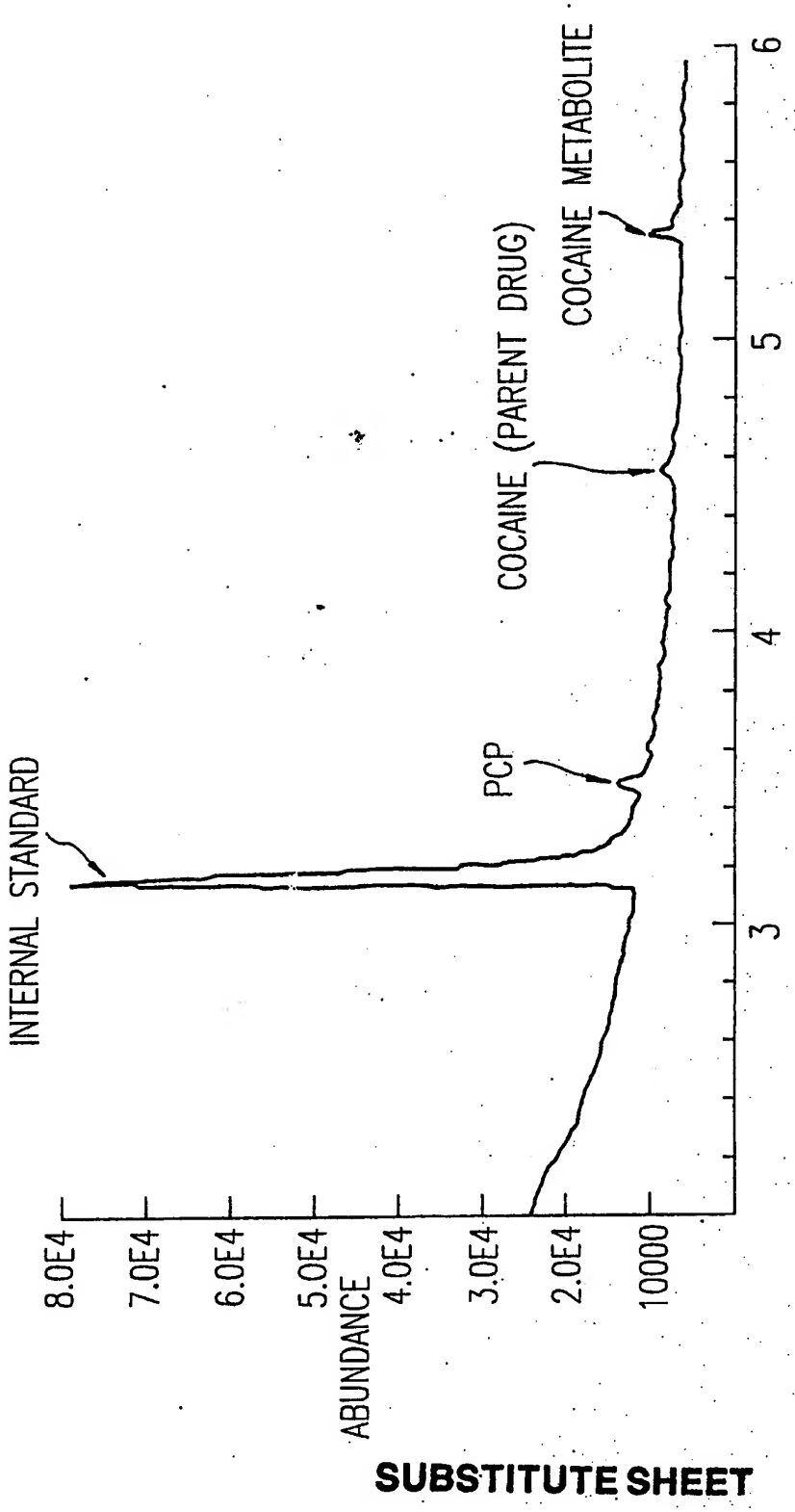
INTERNAL STANDARD
PCP
COCAINE (PARENT DRUG)
COCAINE METABOLITE
8.0E4
7.0E4
6.0E4
5.0E4
4.0E4
3.0E4
2.0E4
10000
ABUNDANCE
3
4
5
6
TIME (MINUTES)

FIG. 2A

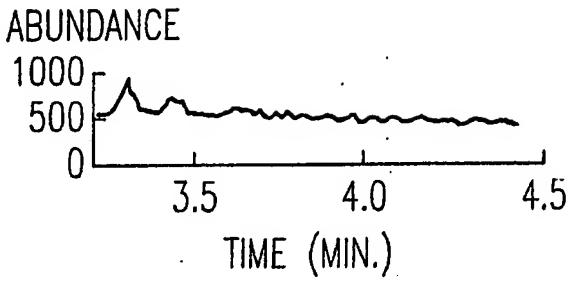

FIG. 2B

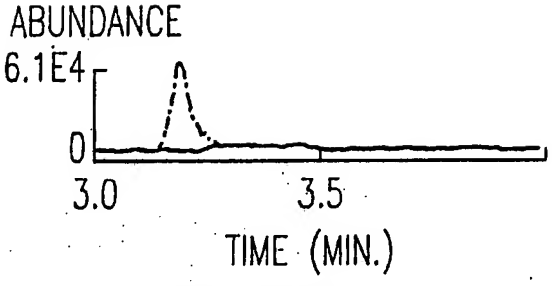

FIG. 2C

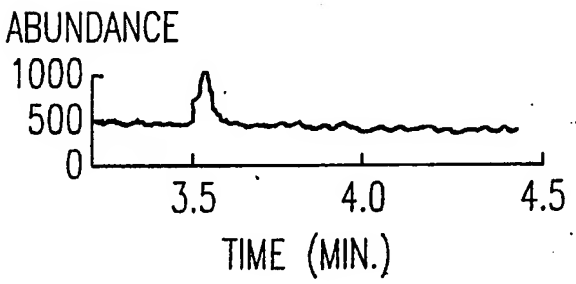

FIG. 2D

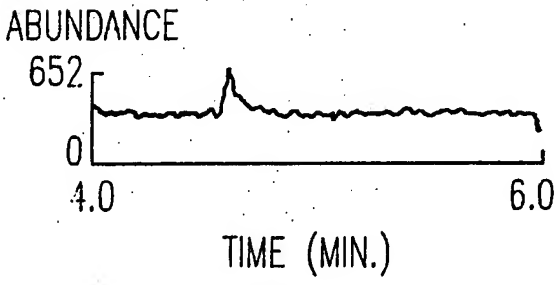

FIG. 2E

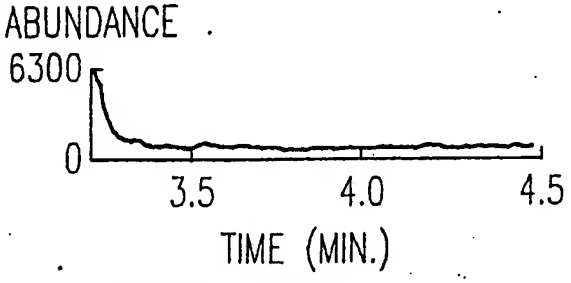

FIG. 2F

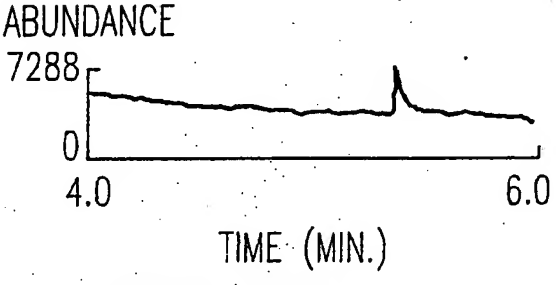

FIG. 2G

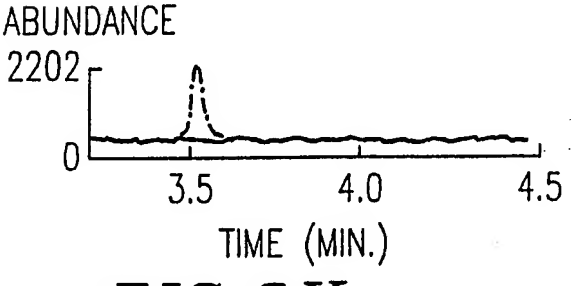

FIG. 2H

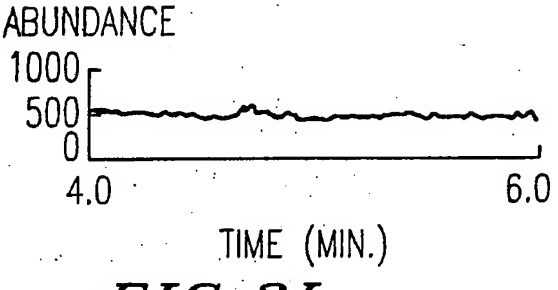

FIG. 2I

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US90/06657

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): G01N 33/535, 33/567, 30/02, 24/00

U.S. Cl.: 435/7.9; 436/161, 173, 504

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/7.1, 7.9, 975; 436/161, 173, 503, 504, 518, 548, 804, 808, 815, 816, 822; 128/757, 759

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Chemical Abstracts Services Online (File CA, 1967-1991, file Biosis Preview, 1969-1991) Automated Patent System (File USPAT, 1975-1991). See attachment for search terms.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 3,965,888 **(BENDER)** 29 JUNE 1976, see column 1, lines 31-37, lines 56-63, Figure 6.	1-9
Y	US, A, 4,771,005 **(SPIRO)** 13 SEPTEMBER 1988, see column 4, lines 29-36.	1-4, 9
Y	JOURNAL OF CLINICAL CHEMISTRY AND CLINICAL BIOCHEMISTRY, Volume 27, No. 11, issued October 1989. **H. SACHS**, "Results of Comparative Determination of Morphine in Human Hair using RIA and GC/MS" pages 873-877, see page 876, column 1, next to the last paragraph, column 2, final paragraph.	1 - 5
Y	US, A, 4,495,281 **(BUCKLER ET AL.)** 22 JANUARY 1985, see column 10, line 33 to column 11, line 3.	6, 8, 9
Y	Biological Abstracts, Volume 88, No. 6, issued 15 SEPTEMBER 1989, **OFFIDANI ET AL.**, "Drugs in hair: A new extraction procedure," Sci. Int., 41(1/2), 35-40,see page 1243, column 1, abstract no. 69055.	4

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search: 28 January 1991

Date of Mailing of this International Search Report: 08 MAR 1991

International Searching Authority: ISA/US

Signature of Authorized Officer: Carol E Bidwell

Carol E. Bidwell (vsh)

Form PCT/ISA/210 (second sheet) (rev. 11 87)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	Chemical Abstracts, Volume 108, No. 21, issued 23 MAY 1988, **NANJI ET AL.**, "Detection of drugs in patients with overdose: comparison between skin surface air sampling and thin layer chromatography," see page 218, column 2, abstract no. 181598t, Int. J. Clin. Pharmacd., Ther. Toxical. 26(1) 1-3.	1-8

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers , because they relate to subject matter [2] not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [3], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ATTACHMENT TO FORM PC/ISA/210, PART II.

II. FIELDS SEARCHED/SEARCH TERMS:

Drug#

Nail#

Fingernail#

Skin

(Illegal or cocaine or PCP or hashish or mari?una)

Stratum corneum

Adhesive (20a) (collect? or sampl? or skin dermis)